under the control of tissue specific promoter elements as well as a variety of viral and mammalian constitutive promoter elements.

Problems associated with adenovirus infection, particularly those associated with repression of host cell mRNA translation and shutdown of host normal mRNA production have been addressed by using defective adenovirus vectors which are based on mutations in the dominant regulatory region, E1. In addition, conventional adenovirus vector systems typically require high cell exposure for expression of the desired gene, which is detrimental to the cells because of cytopathic effects from exposure. Therefore, a need exists for an adenovirus-mediated expression vector which can infect cells at low doses, yet can exhibit maximum expression of a gene in the cell.

Assays using 1) a cell system that permits intracellular replication of the plasmid vector during transient expression studies; or 2) transfectants that stably express the receptor of interest, provide useful, but, limited receptor expression. Where transfections yield low levels of receptor expression, or where the range of cell types that can be transfected is restricted, studies of these receptors is limited. Adenovirus-mediated gene transfer could be employed as an alternative strategy to plasmid based receptor expression vectors. A significant advantage of using adenovirus-mediated gene transfer is the wide variety of cells which are susceptible to infection by adenovirus. This should permit study of TRH-R biology in a variety of mammalian cell types, including those not amenable to transfection techniques.

The present invention is directed to overcoming these deficiencies in the art.

The rejection of claim 6 under 35 U.S.C. § 112 (1st and 2nd paras.) is respectfully traversed in view of the above amendments cancelling claim 6.

The rejection of claims 10-12 and 14 under 35 U.S.C. § 112 (1st and 2nd paras.) is respectfully traversed in view of the above amendments.

The rejection of claims 1-17 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in the above amendments.

The rejection of claim 12 under 35 U.S.C. § 102 or 35 U.S.C. § 103 as anticipated by or obvious over Quantin, et. al., "Adenovirus as an Expression Vector in Muscle Cells *in vivo*," Proc. Natl. Acad. Sci. USA 89: 2581-84 (1992)("Quantin") or Stratford-Perricaudet, et. al., "Widespread Long-Term Gene Transfer to Mouse Skeletal

Muscles and Heart," J. Clin. Invest. 90: 626-30 (1992)("Stratford-Perricaudet") is respectfully traversed in view of the cancellation of claim 12.

The rejection of claims 1-17 under 35 U.S.C. § 103 for obviousness over Kirshenbaum, et. al., "Highly Efficient Gene Transfer into Adult Ventricular Myocytes by Recombinant Adenovirus," J. Clin. Invest. 92: 381-87 (1993)("Kirshenbaum"), Quantin, or Stratford-Perricaudet in view of Huang, et. al., "Intervening Sequences Increase Efficiency of RNA 3' Processing and Accumulation of Cytoplasmic RNA," Nucl. Acids Res. 18(4): 937-47 (1990)("Huang"), Keating, et. al., "Effect of Different Promoters on Expression of Genes Introduced into Hematopoietic and Marrow Stromal Cells by Electroporation," Exp. Hematol. 18: 99-102 (1990)("Keating"), and WO 91/00747 to Kabigen AB ("Kabigen") is respectfully traversed.

Kirshenbaum discloses a recombinant adenovirus vector for gene transfer into ventricular myocytes (Declaration of Erik S. Falck-Pedersen Under 37 CFR § 1.132 ("Falck-Pedersen Declaration") ¶ 5). This vector utilizes, in order, part of the adenovirus type 5 genome, a human cytomegalovirus immediate-early promoter, an *E. coli* lacZ gene, an SV40 polyadenylation signal, and part of the adenovirus type 5 genome (Id.). Unlike the claimed invention, there is no eukaryotic splice acceptor and splice donor downstream of the promoter.

Quantin discloses a recombinant adenovirus expression vector for gene transfer to muscle cells (Falck-Pedersen Declaration ¶ 6). This vector contains, in order, a portion of the adenovirus 5 genome, an enhancer fragment of the mouse myosin light chain 1/3 locus, the mouse skeletal α-actin gene promoter, the β-galactosidase gene, the polyadenylation from simian virus, and part of the adenovirus 5 genome (Id.). Like Kirshenbaum (and unlike the present invention), Quantin does not have a eukaryotic splice acceptor and splice donor downstream of the promoter.

Stratford-Perricaudet discloses a recombinant adenovirus expression vector for gene transfer to mouse skeletal muscles and heart (Falck-Pedersen Declaration ¶ 7). In sequence, this vector includes a portion of an adenovirus 5 genome, the Rous sarcoma virus long terminal repeat, the β-galactosidase gene, the polyadenylation sequence from simian virus 40 early region, and part of the adenovirus genome 5 (Id.). Again, there is no eukaryotic splice acceptor and splice donor downstream of the promoter.

In Huang, expression vectors for gene transfer into mammalian cells are described. The pMLSS.CAT vector includes, in series, a simian virus 40 early promoter with enhancer, an adenovirus major late region, an IgG variable region which forms a splice acceptor region, a chloramphenicol acetyl transferase encoding gene, and a late polyadenylation site (Falck-Pedersen Declaration ¶ 9). There are no left end replication and package elements of the adenovirus 5 genome upstream of the promoter nor an adenovirus 5 genome region positioned downstream of an insertion site (Id.). Accordingly, the plasmid of Huang is not capable of forming a recombinant adenovirus (Id.).

Keating relates to the effect of different promoters on expression of genes introduced into hematopoietic and marrow stromal cells. As to the present invention, Keating does not cure the above-noted deficiencies of Kirshenbaum, Stratford-Perricaudet, Quantin, or Huang.

Kabigen discloses an expression vector containing 2 protein-encoding DNA fragments. Kabigen does not obviate the deficiencies of Kirshenbaum, Stratford-Perricaudet, Quantin, Huang, or Keating with respect to the present invention.

It would not have been obvious to combine the teachings of Kirshenbaum, Stratford-Perricaudet, or Quantin with that of Huang. Huang has nothing to do with a recombinant adenovirus expression system. Although Huang does indicate an improvement in expression due to the presence in the disclosed vector of a splicing element, there is no basis for concluding that such an effect would be achieved in the recombinant adenovirus expression systems of Kirshenbaum, Stratford-Perricaudet, or Quantin. Adenovirus has duplex linear DNA of 36 kilobases and is non-covalently bound to a nuclear matrix through a terminal protein. This platform for transgene expression is very different than a plasmid vector. More particularly, a plasmid vector is a circular duplex DNA which is episomal and a free floating DNA template. In view of these differences, one of ordinary skill in the art would have had no motivation to adapt the teachings of Huang's plasmid vector to the adenovirus systems of Kirshenbaum, Stratford-Perricaudet, and Quantin. Adenovirus has duplex linear DNA of 36 kilobases and is non-covalently bound to a nuclear matrix through a terminal protein. This platform for transgene expression is very different than a plasmid vector. More particularly, a plasmid vector is a circular duplex DNA which is episomal and a free floating DNA template. In view of these differences, one of ordinary skill in the art would have had no motivation to adapt the teachings of Huang's plasmid vector to the

adenovirus systems of Kirshenbaum, Stratford-Perricaudet, and Quantin. Similarly, although Keating teaches that the CMV promoter achieves a higher level of expression when a vector containing it and a gene to be expressed are electroporated into stromal cells, there is no reason to expect such a result in a recombinant adenovirus system. Therefore, one of ordinary skill in the art would have no reason to utilize a splicing element or CMV promoter in the vectors of Kirshenbaum, Stratford-Perricaudet, or Quantin. In view of this lack of combinability of the cited references, the obviousness rejection cannot be properly maintained.

Even if, assuming *arguendo*, the cited references were combinable, which they are not, any *prima facia* case of obviousness would be rebutted by the evidence of unexpected results set forth in the Falck-Pedersen Declaration. See In re De Blauwe, 736 F.2d 699, 222 USPQ 191 (Fed. Cir. 1984). More particularly, a comparison of the results in Figure 2 for the pMLSISCATL3dISS vector versus those achieved with the other vectors tested demonstrates that substantially greater amounts of acetylated product are achieved when a vector has a splice and donor site. Thus, the vector of the present invention achieves significantly better expression than do the vectors of Quantin, Stratford-Perricaudet, or Kirshenbaum which lack such a site. (Id. ¶ 12). As to the significance of utilizing the vector of the present invention over that of Huang, a comparison of the results in Figure 2 for the pMLSISCAT vector versus the results for the pAdCMVCATgD vector shows that much higher levels of expression are achieved with the present invention than with Huang's vector (Id. ¶ 13). Thus, the present invention achieves substantially greater expression than any of Quantin, Straford- Perricaudet, Kirshenbaum, or Huang (Id. ¶ 14). In view of these results, the rejection based upon these references in combination with Keating and Kabigen should be withdrawn.

In view of all the foregoing, it is respectfully submitted that this case is in condition for allowance, and such allowance is earnestly solicited.

Respectfully submitted,

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